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Microcyclospora rumicis, a new species on *Rumex crispus* from Iran

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Abstract — A new species of *Microcyclospora,* associated with leaf spots on *Rumex crispus*, is fully described and illustrated.

Key words — hyphomycetes, *Pseudocercospora*, weed, cercosporoid fungi

Introduction

During the study of alternative weed hosts for *Cercospora beticola* Sacc., a causal agent of cercospora leaf spot of sugar beet in Northern Iran, a species of the genus *Microcyclospora* J. Frank et al. was isolated from *Rumex crispus* showing leaf spot symptoms in the Talesh region (Guilan province). *Microcyclospora,* with *M. pomicola* J. Frank et al. designated as type species, was recently segregated from *Pseudocercospora* Speg. s. str. based on phylogenetic and morphological differences (Frank et al. 2010). Currently the three species known in *Microcyclospora* are associated with sooty blotch and flyspeck (SBFS) blemishes on the surfaces of pomaceous fruits, specifically apples (Colby 1920; Frank et al. 2010). Here we describe a new *Microcyclospora* species from leaf spots on *Rumex crispus* that differs morphologically from the three other known species of the genus.

Materials & methods

Conditions of isolation, culture, and observation

Leaf samples of *Rumex crispus* showing leaf spot were collected from Rainforest Mountains in the Talesh region, Guilan province, in northern Iran near the Caspian Sea. Single conidial isolates were established directly from symptomatic curly dock leaves according to Bakhshi et al. (2011). A mass of conidia scraped from the lesion using a sterile inoculation needle under a stereomicroscope was floated in 10 ml sterile distilled water and spread on 2% malt extract agar (MEA; Fluka, Germany). After plates

were incubated in a slanted position overnight, germinated conidia were transferred to new MEA plates, and cultures were incubated in the dark at 25 °C. After 30 days of incubation, samples from the colony were mounted on glass slides in clear lactic acid for microscopic examination. Thirty measurements were made for each microscopic structure, and 95% confidence intervals were derived for the measurements with extreme values given in parentheses. Line drawings were prepared using a BX41 light microscope (Olympus, Japan) equipped with a drawing tube; photos were captured using a Leica camera system. Colony colors on MEA and oatmeal agar (OA; Gams et al. 2007) [surface and reverse] were determined after one month at 25 °C in the dark. Nomenclature and descriptions were deposited in MycoBank. The holotype voucher and an ex-type culture are conserved in CCTU, the culture collection housed at the Plant Protection Department, Agriculture Faculty, University of Tabriz, Iran.

Taxonomy

Microcyclospora rumicis Arzanlou & Bakhshi, **sp. nov.** Plate 1–2

MycoBank MB 563723

Microcyclospora rumicis *ab aliis speciebus generis chlamydosporis numerosis, majoribus, ad 20 µm diam distinguenda.*

Type: Iran, Guilan, Talesh, on leaves of *Rumex crispus* L. (*Polygonaceae*), Sep. 2010, M. Bakhshi (**Holotype**, CCTU-H-1; **ex-type culture**: CCTU 1 = CBS 131546).

Etymology: Named after the host genus, *Rumex.*

Culture characteristics — On MEA slow growing, reaching 3 mm diam after 7 d, and up to 10 mm after 2 weeks at 25 °C, raised, unevenly folded, with moderate, smoke-gray aerial mycelium; surface irregular, with smooth, lobate margins, iron-grey; iron-grey in reverse. Colonies on OA reaching up to 2 mm diam after 7 d, and 6 mm after 2 weeks at 25 °C, flat, submerged, with sparse aerial mycelium and smooth margin. Microcyclic conidiation commonly observed on all media in culture.

In vitro on MEA — Mycelium consisting of branched, smooth, septate, pale brown, 2–4 µm wide hyphae developing numerous chlamydospores, intercalary and terminal, medium brown, 6–20 µm diam. Conidiophores reduced to conidiogenous cells, integrated, lateral on hyphae, mono- to polyblastic, subdenticulate, 2.5–4 µm wide, 8–13 µm tall, hyaline, smooth. Conidia scolecosporous, cylindrical, straight to variously curved, guttulate, apex obtuse, base truncate, hyaline, (1.5–)2.5(–4) × (15–)37–54(–100) µm, 1–10-septate; hila neither thickened nor darkened; microcyclic conidiation commonly observed; older conidia developing intercalary chlamydospores that are pale brown, ≤ 9 µm diam.

Discussion

The cercosporoids are amongst the major groups of fungi and generally cause leaf spot diseases on almost all families of flowering plants throughout

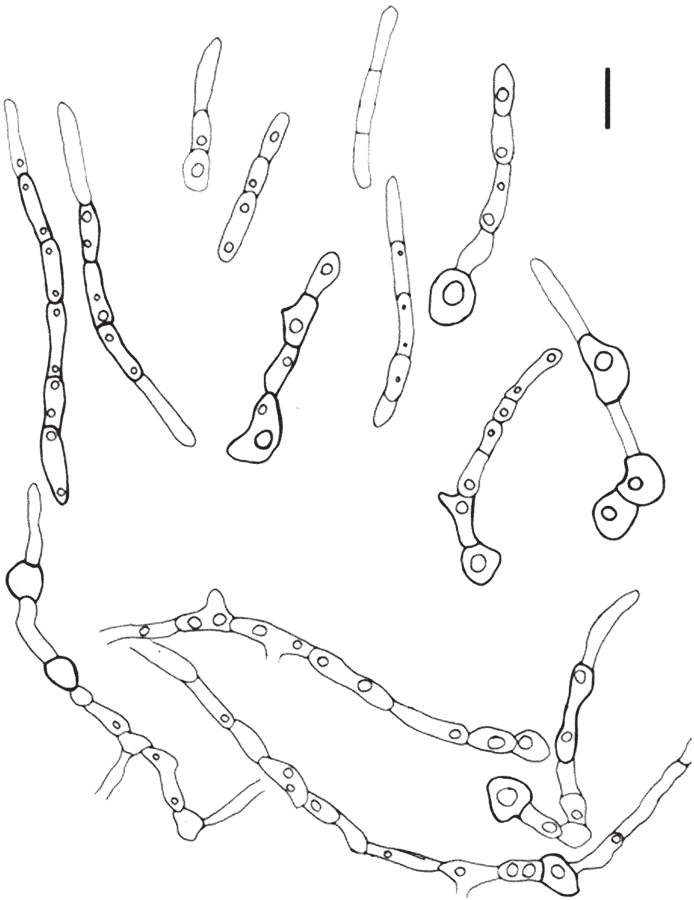

Plate 1. *Microcyclospora rumicis.*
Conidia, conidiogenous cells and hyphae from MEA colony. Bar = 10μm.

the world. Among these, *Pseudocercospora* is the second largest cercosporoid genus, with more than 300 published names (Kirk et al. 2008). Recently it was shown that *Pseudocercospora* included taxa that vary considerably in their conidiomatal morphology, ranging from solitary conidiogenous loci, synnemata, sporodochia to fascicles. Moreover, conidia in some taxa are transversely euseptate but with some oblique and longitudinal septa or containing a mixture of eu- and distoseptation. Conidial hila and scars vary from unnoticeable to slightly thickened along the rim (Stewart et al. 1999). Even though the conidia

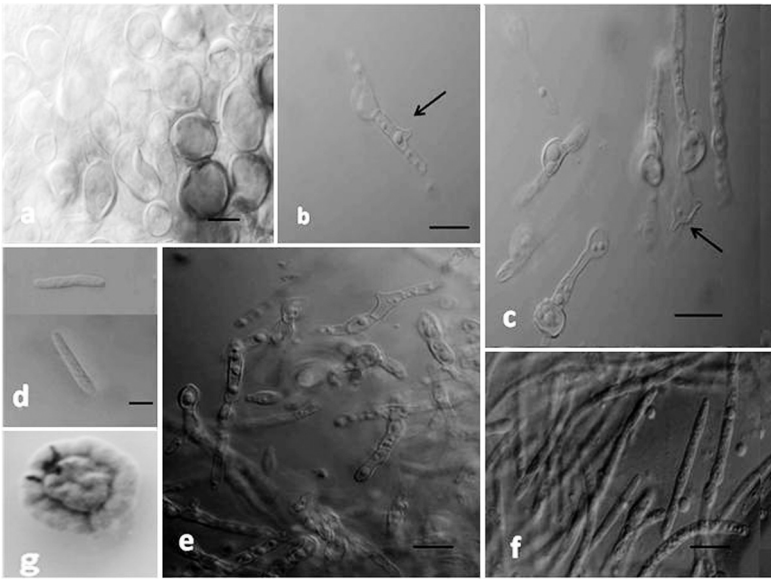

Plate 2. *Microcyclospora rumicis*. a. Chlamydospore in hyphae; b–c. Conidiogenous loci (arrowed) and conidia with chlamydospore; d. Young conidia; e–f. Hyphae. g. 30-day old colony on MEA. Scale bars = 10 μm.

are commonly solitary, they may in some cases also occur in unbranched chains (Braun 1995). Frank et al. (2010) established *Microcyclospora* based on morphological and molecular data. Morphologically, *Microcyclospora* can be distinguished from *Pseudocercospora* by the arrangement of the conidiophores that are never fasciculate but reduced to solitary conidiogenous loci on hyphae and conidia that aggregate in mucoid masses, strongly tending toward microcyclic conidiation. Only three other species described in the genus —*M. malicola* J. Frank et al., *M. pomicola,* and *M. tardicrescens* J. Frank et al.— associated with sooty blotch and flyspeck (SBFS) blemishes on surfaces of pomaceous fruits (Frank et al. 2010). Here, we introduce a new species based on morphological characteristics. *Microcyclospor*a *rumicis,* like *M. tardicrescens,* forms intercalary chlamydospores (lacking in *M. malicola* and *M. pomicola*) and grows more slowly in culture. Chlamydospores of *M. rumicis* are longer (≤ 20 μm) than those of *M. tardicrescens* (≤ 5 μm long).

Little is known about sexual stages of the cercosporoid fungi including *Microcyclospora* species. Cercosporoids have been traditionally treated as anamorphs of the ascomycete *Mycosphaerella* Johanson (e.g., Braun & Mel'nik 1997, Kim & Shin 1998, Crous & Braun 2003). However, most cercosporoids

are considered exclusively asexual, and *Mycosphaerella* teleomorphs have been confirmed for only a few species.

Phylogenies derived from multiple sequence analyses place *Microcyclospora* with other cercosporoid fungi in the *Mycosphaerella* clade (*Mycosphaerellaceae*, *Capnodiales*, *Dothideomycetidae*; Frank et al. 2010). *Mycosphaerella,* one of the largest genera in the *Ascomycota,* comprises several thousand species (Crous et al. 2001, 2009a,b; Aptroot 2006). Contrary to the assumption that *Mycosphaerella* as then defined was monophyletic, Crous and co-workers recently revealed that the genus is polyphyletic and split the former *Mycosphaerellaceae* into several families, of which the *Mycosphaerellaceae*, *Teratosphaeriaceae* and *Schizothyriaceae* have plant-pathological importance (Crous et al. 2007, 2009a). Among the vast number of anamorphs in these lineages, up to 30 anamorph genera have been linked to *Mycosphaerella* (Crous et al. 2007, 2009a; Arzanlou et al. 2007). Recent phylogenetic analysis based on multiple sequence data sets, however, indicate that these interpretations are not quite correct (Crous et al. 2009b) and that the *Mycosphaerellaceae* encompass instead numerous genera with morphologically conserved *Mycosphaerella*-like teleomorphs but quite distinct anamorphs (Crous et al. 2007, 2009b).

Little is currently known about ecology, host ranges, and sexual stages of the cercosporoid fungi including *Microcyclospora* species, and more sampling on different substrates is needed to address these aspects.

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